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Patricia A. Johnson

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IN THE UNITED STATES PATENT AND TRADEMARK C[illegible]

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In the application of:

James C. Chen

Serial No.: 09/760,362

Filing Date: Jan. 12, 2001

For: NOVEL TREATMENT FOR EYE DISEASE

Examiner: To Be Ass[illegible]

Group Art Unit: 1614

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please replace the original specification with the attached replacement specification. The amendments to the specification transfer text found in the original drawings to the specification and replace line numbering with paragraph numbering, and consequently the amendments add no new matter to the application.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached marked-up copy of the specification is captioned "**Version with markings to show changes made**".

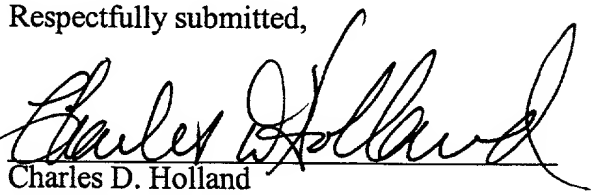
Also enclosed are substitute drawings in compliance with 37 CFR 1.84.

pa-638832

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 398032000900.

Respectfully submitted,

Dated: October 31, 2001

By: 

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DETAILED DESCRIPTION OF THE INVENTION

[031] This invention provides methods for treating neovascular disease of the eye by the specific and selective binding of a photosensitizing compound to the abnormal endothelium that lines or composes the neovasculature target tissue. This method comprises illuminating the photosensitized target tissue with light for a period of time sufficient to activate the bound photosensitizing compound thereby causing damage to the neovasculature target tissue.

[032] Specifically, the present invention is based on the precise targeting of photosensitizing compounds to specific target receptors and/or antigens present on abnormal endothelium or to specific ligands and/or antibodies which are themselves bindable to endothelial receptors and antigens, and to the method of activation of the bound and targeted photosensitizing compounds by subsequently administering to the target tissue light of a relatively low fluence rate over a prolonged period of time. The disclosed method achieves maximal damage to abnormal endothelium with minimal side effects or collateral tissue damage.

[033] Terms as used herein are based upon their art recognized meaning and from the present disclosure should be clearly understood by the ordinary skilled artisan. For sake of clarity, terms may also have particular meaning as would be clear from their use in context.

[034] Further, as used herein, "target tissues" are those tissues that are intended to be impaired or destroyed by this treatment method. Photosensitizing compounds bind to these target tissues; then when sufficient radiation is applied, these tissues are impaired or destroyed.

[035] "Non-target tissues" are all the tissues of the eye which are not intended to be impaired or destroyed by the treatment method. These non-target tissues include but are not limited to healthy blood cells, and other normal tissue of the retina and choroid, not otherwise identified to be targeted.

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[036] "Photosensitizing compound" is a chemical compound which homes to one or more types of selected target cells and, when contacted by radiation, absorbs the light, which results in impairment or destruction of the target cells. Virtually any chemical compound that homes to a selected target and absorbs light may be used in this invention. Preferably, the chemical compound is nontoxic to the subject to which it is administered or is capable of being formulated in a nontoxic composition. Preferably, the chemical compound in its photodegraded form is also nontoxic. A comprehensive listing of photosensitive chemicals may be found in Kreimer-Birnbaum, Sem. Hematol. 26:157-73, 1989. Photosensitive compounds include, but are not limited to, chlorins, bacteriochlorophylls, phthalocyanines, porphyrins, purpurins, merocyanines, psoralens, benzoporphyrin derivatives (BPD) and porfimer sodium and pro-drugs such as δ-aminolevulinic acid, which can produce drugs such as protoporphyrin. Other compounds include indocyanine green (ICG); methylene blue; toluidine blue; texaphyrins; and any other agent that absorbs light in a range of 500 nm -1100 nm.

[037] "Illumination" as used herein includes all wave lengths and wavebands. Preferably, the illumination wave length or waveband is selected to match the wave length(s) or wavebands which excite the photosensitive compound. Even more preferably, the radiation wave length or waveband matches the excitation wave length or waveband of the photosensitive compound and has low absorption by the non-target tissues of the eye, and the rest of the subject, including blood proteins.

[038] The irradiation by illumination is further defined in this invention by its coherence (laser) or non-coherence (non-laser), as well as intensity, duration, and timing with respect to dosing using the photosensitizing compound. The intensity or fluence rate must be sufficient for the light to reach the target tissue. The duration or total fluence dose must be sufficient to photoactivate enough photosensitizing compound to act on the neovasculature target tissue. Both intensity and duration must be limited to avoid overtreating the subject. Timing with respect to dosing with the photosensitizing compound is important, because 1) the administered

photosensitizing compound requires some time to home in on neovasculature target tissue and 2) the blood level of many photosensitizing compounds decreases with time.

[039] Briefly, the photosensitizing compound is generally administered to the subject before the neovasculature target tissue is subjected to illumination.

[040] Preferred photosensitizing compounds include, but are not limited to, chlorins, bacteriochlorophylls, phthalocyanines, porphyrins, purpurins, merocyanines, psoralens and pro-drugs such as δ-aminolevulinic acid, which can produce drugs such as protoporphyrin. More preferred are: methylene blue; toluidine blue; texaphyrins; and any other agent that absorbs light in a range of 600 nm -1100 nm. Most preferred is indocyanine green (for example, see: WO 92/00106 (Raven *et al.*); WO97/31582 (Abels *et al.*) and Devoisselle *et al.*, *SPIE* 2627:100-108, 1995). Additional photosensitizing compounds, include: pyropheophorbide compounds (see: U.S. Patent No.: 5,459,159); bacteriochlorophyll derivatives (see: U.S. Patent No.: 5,955,585); and Alkyl ether analogs of chlorins (see: U.S. Patent No.: 5,952,366).

[041] Any one or combination of these or other photosensitizing compounds may be supplied in a kit of this invention along with instructions on conducting any of the methods disclosed herein. Instructions may be in any tangible form, such as printed paper, a computer disk that instructs a person how to conduct the method, a video cassette containing instructions on how to conduct the method, or computer memory that receives data from a remote location and illustrates or otherwise provides the instructions to a person (such as over the Internet). A person may be instructed in how to use the kit using any of the instructions above or by receiving instructions in a classroom or in the course of treating a patient using any of the methods disclosed herein, for example.

[042] The photosensitizing compound is administered orally, intravenously by injection, or via the intraocular route. The photosensitizing compound can be conjugated to various antibodies, antibody fragments, and other molecules and

compounds capable of binding to the endothelium of neovessels. The specific ligands reactive with the target endothelium include antibodies and antibody fragments that bind to abnormal or upregulated vascular endothelial receptors such as the VEGF receptors and α-3, β-3 integrins (see: Ferrara, *Curr Top Microbiol Immunol*, 237:1-30, 1999; Elicieri and Cheresh, *The Journal of Clinical Investigation*, 103:1227-30, 1999; Smith *et al.*, *Br J Opthamol*, 83:486-494, 1999). Also, the antibody can be drawn to and have affinity to bind to the extra-domain B (or ED-B) of fibronectin. Such antibodies, include a complete or functional bindable fragment of a human antibody, such as L19 or its equivalent (see: Birchler *et al.*, *Selective targeting and photocoagulation of ocular angiogenesis mediated by a phage-derived human antibody fragment*, *Nature Biotech.* 17: 984 (1999)). The ligand can be any molecule or compound that binds to a endothelial receptor found on an abnormal blood vessel wall. Preferably the ligand binds selectively to receptors which are mainly or only found on the abnormal blood vessel wall.

[043] Another embodiment of the present invention involves the use of a photosensitizing compound bound to a receptor-type molecule or compound. The receptor mimics the type of receptors found on the endothelium of abnormal vessel walls. Preferably the receptor mimic binds ligands, such VEGF, that are found to be elevated in concentration or are not normally present due to the abnormal conditions relating to the abnormal blood vessel formation. An additional embodiment involves the use of a bispecific antibody construct that is a combination ligand and receptor type molecule or compound that is bound to a photosensitizing compound. The bispecific nature of this construct allows binding of either an abnormal endothelial receptor or an abnormal ligand or abnormally elevated concentration of ligand.

[044] Alternatively, the photosensitizing compound can be packaged into liposomes and the ligand, receptor, or bispecific construct incorporated or attached to the liposome to serve as a further means of targeting. In each of the above embodiments,

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preferably more than one photosensitizing compound is attached to the targeting moiety.

[045] The technique of constructing bispecific antibodies, the techniques and methods of linking photosensitizers to targeting agents, and the techniques of producing targeted liposomes are well known in the art. For example, useful reviews of such techniques are provided by Yatvin *et al.*, U.S. Patent No. 5,827,819 (1998) and Jansen , *et al.*, U.S. Patent No. 5,869,457 (1999).

[046] The bound photosensitizing compound can be administered in a dry formulation, such as pills, capsules, suppositories or patches. The compound also may be administered in a liquid formulation, either alone with water, or with pharmaceutically acceptable excipients, such as are disclosed in Remington's Pharmaceutical Sciences. The liquid formulation also can be a suspension or an emulsion. In particular, liposomal or lipophilic formulations are desirable. If suspensions or emulsions are utilized, suitable excipients include water, saline, dextrose, glycerol, and the like. These compositions may contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, antioxidants, pH buffering agents, and the like.

[047] The dose of photosensitizing compound can be determined clinically and will be the lowest dose that saturates the available binding sites. Depending on the photosensitizing compound used, an equivalent optimal therapeutic level will have to be established. A certain length of time is allowed to pass for the circulating or locally delivered photosensitizer to be taken up by the endothelium of the neovessels. The unbound photosensitizer is cleared from the circulation during this waiting period. The waiting period will be determined clinically and may vary from compound to compound.

[048] At the conclusion of this waiting period, a non-laser light source is used to activate the bound drug, although a laser light source may be used. The spot size illuminating the retina or choroid is determined by the location and dimension of the

pathologic region to be treated. The duration of illumination period will be determined empirically, but is preferably a total or cumulative period of time between about 4 minutes and 72 hours. More preferably, the illumination period is between about 60 minutes and 148 hours. Most preferably, the illumination period is between about 2 hrs and 24 hours.

[049] Preferably, the total fluence or energy of the light used for irradiating, as measured in Joules, is between about 30 Joules and about 25,000 Joules; more preferably, between about 100 Joules and about 20,000 Joules; and most preferably, between about 500 Joules and about 10,000 Joules. Light having a waveband corresponding at least in part with the characteristic light absorption waveband of said photosensitizing agent is used for irradiating the target tissue.

[050] The intensity or power of the light used is measured in watts, with each Joule equal to one watt-sec. Therefore, the intensity of the light used for irradiating in the present invention may be substantially less than 500 mW/cm^2. Since the total fluence or amount of energy of the light in Joules is divided by the duration of total exposure time in seconds, the longer the amount of time the target is exposed to the irradiation, the greater the amount of total energy or fluence may be used without increasing the amount of the intensity of the light used. The present invention employs an amount of total fluence of irradiation that is sufficiently high to activate the photosensitizing agent, as applicable, with a concomitant reduction in the intensity of light and collateral or non-target specific tissue damage.

[051] While not wishing to be limited by a theory, the inventor proposes that a targeted photosensitizing compound can be substantially and selectively photoactivated in the neovasculature target tissue within a therapeutically reasonable period of time and without excess toxicity or collateral damage to non-target tissues. A relatively low fluence can be used for a relatively long period of time in order to fully photoactivate the drug in order to insure adequate closure of the neovessels and vessel abnormalities.

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[052] Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLES

EXAMPLE 1 TREATMENT OF CHOROIDAL NEOVASCULATURE LESIONS

[053] A subject with choroidal neovascularization (CNV) from age-related macular generation is assessed using standard visual acuity testing, ophthalmic examination, color photographs and fluorescein angiograms (see Miller *et al.*, *Ach. Ophthal.* vol. 117:1161-1173 (1999)).

[054] A photosensitizing agent, verteporfin, is conjugated using generally recognized methods in the art to a bindable fragment of the L19 antibody demonstrating high affinity to the ED-B of fibronectin (Birchler *et al., Nature Biotech.* 17: 984 (1999)). A therapeutically effective amount of the photosensitizing agent conjugate, approximately 5 mg/m^2, is administered intravenously to the subject.

[055] Following a period of approximately 1 hour, to permit the non-specifically bound photosensitizing agent conjugate to clear from collateral tissues, the subject is irradiated in one or more sessions for a total period of 10 minutes with 400 mW/cm^2 of collimated LED light having a wavelength of 690 nm. This represents a total fluence of 240 $Joules/cm^2$.

[056] The entire lesion is treated with a single spot of the size as determined from a pretreatment angiogram. A margin of 300-500 μm may be added to ensure complete coverage of the lesion. A green non-activating observation light beam may be used for real-time observation and aiming during PDT.

[057] Screening examinations may be performed during the first week immediately before treatment. Visual acuity is measured by standard refraction protocol using

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EDTRS criteria. A slit-lamp and a complete ophthalmoscopic exam is performed. Optic discs and maculae of both eyes are documented by stereo color photography. Stereo fluorescein angiography is performed with 10% sodium fluorescein. Frames are taken according to MPS standards. The photodynamic effects are monitored after 1, 4, and 12 weeks by means of visual acuity, ophthalmoscopy, fundus photography and stereo angiography. Angiograms are evaluated for angiographic occlusion and leakage after PDT.

EXAMPLE 2 TREATMENT OF RETINAL NEOVASCULATURE LESIONS

[058] According to Example 1, a liposomal benzoporphyrin derivative is conjugated to VEGF for use as a photosensitizer. A drug dose of 10 mg/m^2 is administered to a subject with neovascular lesions in the retina of the eye via intravenous infusion over 10 minutes. The subject waits for a period of 6 hours to permit clearance from the tissues of non-specifically bound photosensitizing conjugate before illumination therapy is administered.

[059] With the photosensitizer specifically localized to the retinal neovasculature lesions comprising VEGF receptor on the surface of the cells of the lesion, the subject is exposed to non-coherent light from a low power non-coherent broadband light source emitting at 690 nm. This illumination provides a radiant exposure of no more than 500 mW/cm^2 for a period of approximately 20 minutes in one or more sessions producing a total fluence of illumination of about 600 $Joules/cm^2$. Alternatively, coherent or laser light could be similarly employed. Photosensitization is performed with dilated pupils and topical anesthesia using a contact lens.

[060] The entire lesion is treated with a single spot of the size as determined from a pretreatment angiogram. A margin of 300-500 μm is added to ensure complete coverage of the lesion. A green non-activating observation light beam is used for real-time observation and aiming during PDT. Screening examinations and visual acuity as disclosed in Example 1 is performed.

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EXAMPLE 3 TREATMENT OF VASCULAR TUMORS OF THE EYE

[061] Integrin $\alpha v\beta 3$ integrin is expressed by vascular cells during angiogenesis and vascular remodeling and is highly expressed by endothelial cells undergoing angiogenesis in tumors. *See* Eliceiri, B. P. *et al.*, J. Clin. Invest (1999) 103(9):1227-1230. Antibody elicited to $\alpha v\beta 3$, such as LM609 (Vitaxin; Eliceiri *et al.*) is conjugated to a texaphyrin photosensitizing agent in a liposomal formulation. A drug dose of 25 mg/m^2 is administered via intravenous infusion over 10 min. The photosensitizer localizes to the neovasculature lesions. The pupils are dilated to allow ambient light enter for photosensitization. Therefore, no slit lamp is needed for photosensitization and the subject may continue everyday activities while receiving PDT. The ambient light is used to photoactivate the photosensitizing agent for a total exposure time of 24 hours.

[062] Screening examinations and visual acuity as disclosed in Example 1 is performed.

EXAMPLE 4 TREATMENT CHOROIDAL TUMOR OF THE EYE

[063] Most ocular tumors metastasize from systemic origins in breast carcinoma in females, and bronchial carcinoma in males (Chen YR, et al., *Bilateral choroidal metastases as the initial presentation of a small breast carcinoma: a case report*, Chung Hua I Hsueh Tsa Chih (Taipei); 61(2):99-103 1998). Antibody elicited to carcinoembryonic antigen (CEA), which is associated with the choroidal tumor, is conjugated to a benzoporphyrin derivative photosensitizing agent in a liposomal formulation. A drug dose of 10 mg/m^2 is administered via intravenous infusion over 10 min.

[064] Additionally, the patient is administered the anti-$\alpha v\beta 3$ antibody-texaphyrin conjugate at a drug dose of 25 mg/m^2 as provided in Example 3.

[065] After the texaphyrin photosensitizer conjugate localizes to the neovasculature lesion and the benzoporphyrin-anti-CEA conjugate localizes to the CEA tumor

antigens, a period of 6 hours is permitted to pass to permit the unbound or non-specifically bound photosensitizer conjugates to clear from the lesions.

[066] A low power non-coherent broadband light source emitting at 690 nm is used as described in Example 2. The radiant exposure of 250 mW/cm^2 is employed for approximately 1 hour over the course of one or more sessions to provide a total fluence of 900 J/cm^2. Photosensitization is performed with dilated pupils and topical anesthesia using a contact lens. The entire lesion is treated with a single spot of the size as determined from a pretreatment angiogram. A margin of 300-500 μm is added to ensure complete coverage of the lesion. A green non-activating observation light beam is used for real-time observation and aiming during PDT. Screening examinations and visual acuity as disclosed in Example 1 is performed.

[067] Although the present invention has been described in connection with the preferred form of practicing it, those of ordinary skill in the art will understand that many modifications can be made thereto within the scope of the claims that follow. Accordingly, it is not intended that the scope of the invention in any way be limited by the above description, but instead be determined entirely by reference to the claims that follow.

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CLAIMS

The invention claimed is:

1. A method to treat neovascular disease of the eye, comprising:

administering a targeted photosensitizing compound which selectively binds to abnormal endothelium that lines or composes neovasculature tissue; and

illuminating the neovasculature tissue with light for a period of time sufficient to activate the photosensitizing compound thereby causing damage to neovasculature tissue.

2. The method of claim 1, wherein said light is non-laser light.

3. The method of claim 1, wherein said light is laser light.

4. The method of claim 1, wherein the neovasculature tissue is present in retina, choroid or both.

5. The method of claim 1, wherein the treated neovascular disease is diabetic retinopathy.

6. The method of claim 1, wherein the treated neovascular disease is macular degeneration.

7. The method of claim 1, wherein the treated neovascular tissue arises from tumors of the eye.

8. The method of claim 1, wherein said tumors are benign.

9. The method of claim 1, wherein said tumors are malignant.

10. The method of claim 9, wherein said tumors are malignant uveal melanomas.

11. The method of claim 1, wherein the targeted photosensitizing compound is bound to a first component of a bindable pair and wherein a second component of the bindable pair is selected from the group consisting of: receptor present on abnormal endothelium; ligand bindable to receptor present on abnormal endothelium; antigen present on abnormal endothelium; and antibody bindable to antigen present on abnormal endothelium.

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12. The method of claim 11, wherein the targeted photosensitizing compound is incorporated into a liposomal preparation.

13. The method of claim 11, wherein the ligand is selected from the group consisting of: the ED-B domain of fibronectin; antibody specifically elicited to ED-B domain of fibronectin; VEGF; VEGF receptor; and $\alpha v\beta 3$ integrin receptor.

14. The method of claim 1, wherein the targeted photosensitizing compound is bound to a receptor composition that mimics a receptor present on abnormal endothelium.

15. The method of claim 14, wherein the targeted photosensitizing compound is incorporated into a liposomal preparation.

16. The method of claim 1, wherein the targeted photosensitizing compound is bound to a bi-specific antibody construct that further comprises both a ligand component and a receptor component.

17. The method of claim 16, wherein the targeted photosensitizing compound is incorporated into a liposomal preparation.

18. The method of claim 1, wherein the photosensitized neovasculature is illuminated for at least 4 minutes.

19. The method of claim 1, wherein the photosensitized neovasculature is illuminated for at least 20 minutes.

20. The method of claim 1, wherein the photosensitized neovasculature is illuminated for at least 1 hour.

21. The method of claim 1, wherein the photosensitized neovasculature is illuminated for at least 24 hours.

22. The method of claim 1, wherein the neovasculature tissue is treated with a total fluence of light irradiation from between about 240 J/cm^2 to about 900 J/cm^2.

23. The method of claim 1, wherein the non-laser light source is a light emitting diode.

24. The method of claim 1, wherein the non-laser light source is ambient light.

25. A method to treat neovascular disease of the eye, comprising:

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administering a first targeted photosensitizing compound which selectively binds to a first targeted tissue; and

administering a second targeted photosensitizing compound which selectively binds to a second targeted tissue; and

illuminating the first and second targeted tissues with non-laser light for a period of time sufficient to activate said first and second photosensitizing compounds thereby causing damage to said first and second targeted tissue.

26. The method of claim 25, wherein said first targeted tissues is abnormal endothelium that lines or composes neovasculature tissue; and said second targeted tissue is a tumor antigen.

27. The method of claim 26, wherein said first targeted photosensitizing compound comprises a ligand selected from the group consisting of: the ED-B domain of fibronectin; antibody specifically elicited to ED-B domain of fibronectin; VEGF; VEGF receptor; and $\alpha v\beta 3$ integrin receptor.

28. A kit to treat neovascular disease of the eye, comprising a targeted photosensitizing compound and instructions teaching a method according to claim 1.

29. A kit according to claim 28 wherein the targeted photosensitizing compound binds to a first component of a bindable pair and wherein a second component of the bindable pair is selected from the group consisting of: receptor present on abnormal endothelium; ligand bindable to receptor present on abnormal endothelium; antigen present on abnormal endothelium; and antibody bindable to antigen present on abnormal endothelium.

30. A kit according to claim 29, wherein the targeted photosensitizing compound is incorporated into a liposomal preparation.

31. A kit according to claim 29, wherein the ligand is selected from the group consisting of: the ED-B domain of fibronectin; antibody specifically elicited to ED-B domain of fibronectin; VEGF; VEGF receptor; and $\alpha v\beta 3$ integrin receptor.

32. A kit according to claim 28, wherein the targeted photosensitizing compound binds to a receptor composition that mimics a receptor present on abnormal endothelium.

33. A kit according to claim 32, wherein the targeted photosensitizing compound is incorporated into a liposomal preparation.

34. A kit according to claim 28, wherein the targeted photosensitizing compound binds to a bi-specific antibody construct that further comprises both a ligand component and a receptor component.

35. A kit according to claim 34, wherein the targeted photosensitizing compound is incorporated into a liposomal preparation.

36. A method of instructing a person to treat neovascular disease of the eye, comprising instructing a person to conduct a method according to claim 1.

37. A method of instructing a person to treat neovascular disease of the eye, comprising instructing a person in the use of the kit of claim 28.

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ABSTRACT OF THE DISCLOSURE

This invention discloses methods, kits, and instructions to treat neovasculature diseases of the eye through the administration of a targeted photosensitizing agent and subsequent exposure to light of specific wavelength sufficient to photoactivate photosensitizing agent. The photosensitizing agent is bound to a composition that mediates site specific delivery to a neovasculature target tissue of a therapeutically effective amount of a photosensitizing agent that is activated by a relatively low fluence rate of light over a prolonged period of time. Diseases treatable under this invention, include: diabetic retinopathy; macular degeneration; and malignant uveal melanomas.